APPLICATION NOTE

The Chiral Separation of Remdesivir and Several of its Key-Starting Materials

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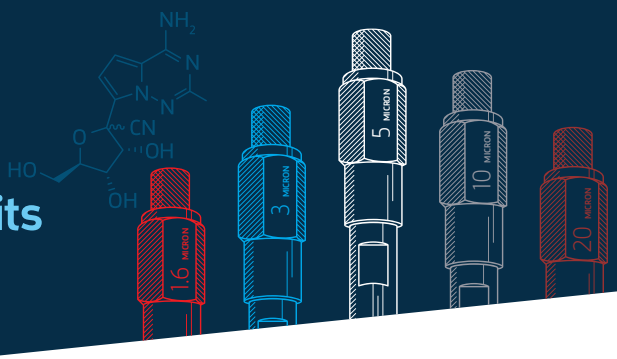

DAICEL Chiral Technologies

INTRODUCTION

The start of 2020 saw the outbreak of the COVID-19 pandemic, and almost immediately, the search for a viable vaccine candidate, and effective treatments for those diagnosed with the virus, began. One of the compounds that emerged early on for the treatment of COVID-19 patients was Remdesivir, an antiviral drug manufactured by Gilead Sciences. Studies conducted since its initial implementation have confirmed its effectiveness in shortening the recovery times of patients hospitalized, and in lowering the overall mortality rate from the disease.

The synthesis of Remdesivir was well documented in the literature, however there are a few key chiral intermediates in the synthesis that were not previously well resolved under high-performance liquid chromatography (HPLC) conditions. The chiral separations of these compounds (shown in Figure 1 along with Remdesivir itself) is hereunder reported on two Daicel immobilized chiral stationary phases (CHIRALPAK® IA-3 and CHIRALPAK® IG-U), as well as on one of Daicel's achiral columns, DCpak® PTZ. The separation of Remdesivir is also reported on CHIRALPAK® IA-3 under normal phase HPLC conditions.

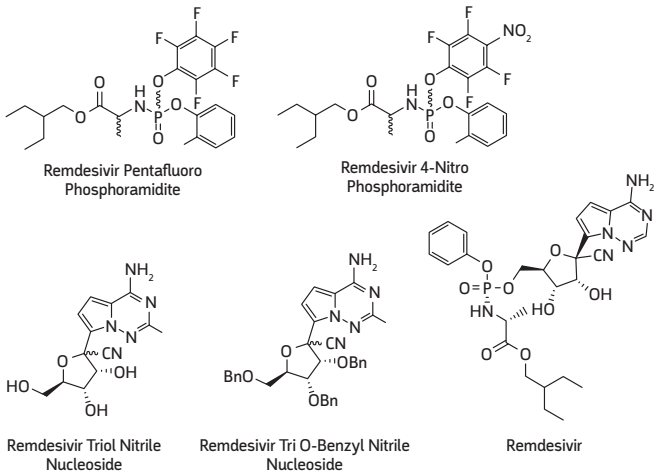

Figure 1

EXPERIMENTAL

Chromatographic Conditions for the Separation of Remdesivir Pentafluoro Phosphoramidite KSM	
Column	CHIRALPAK® IA-3 (250 mm x 4.6 mm i.d.)
Mobile Phase	90-10 = n-Hex-IPA (v/v)
Flow Rate	1.0 ml/min
Detection	210 nm ref. 450 nm
Temperature	25°C
Sample	1.0 mg/ml solution in mobile phase
Injection Volume	10 µl

Chromatographic Conditions for the Separation of Remdesivir 4-Nitro Phosphoramidite KSM	
Column	CHIRALPAK® IG-U (100 mm x 3.0 mm i.d.)
Mobile Phase	Mobile Phase A: Water/Methanol = 5/95 (v/v) Mobile phase B: Acetonitrile Time (min)/%B: 0/0, 3/0, 10/35, 11/0, 16/0
Flow Rate	0.4 ml/min
Detection	270 nm ref. 450 nm
Temperature	40°C
Sample	1.0 mg/ml solution in ACN/Water = 30/70 (v/v)
Injection Volume	3 µl

Chromatographic Conditions for the Separation of Remdesivir Triol Nitrile and Tri O-Benzyl Nitrile Nucleoside KSM	
Column	DCpak® PTZ (150 mm x 4.6 mm i.d.)
Mobile Phase	Mobile Phase A: 0.01% OPA in water Mobile phase B: Methanol/Acetonitrile = 10/90 (v/v) Time (min)/%B: 0/90, 15/50, 20/50, 20.1/90, 25/90
Flow Rate	1.0 ml/min
Detection	245 nm ref. 450 nm
Temperature	40°C
Sample	0.5 mg/ml solution in Methanol/Water = 90/10 (v/v)
Injection Volume	10 µl

Chromatographic Conditions for the Separation of Remdesivir	
Column	CHIRALPAK® IA-3 (250 mm x 4.6 mm i.d.)
Mobile Phase	n-Hexane/Ethanol/IPA/Ethanolamine/Formic Acid = 80/5/15/0.05/0.1 (v/v/v/v/v)
Flow Rate	1.5 ml/min
Detection	245 nm ref. 450 nm
Temperature	40°C
Sample	1.0 mg/ml solution in Hexane/Ethanol = 50/50 (v/v)
Injection Volume	10 µl

DISCUSSION

The two phosphoramidite starting materials contain two chiral centers, one on the phosphorus and the other on the carbon alpha to the phosphoramidite functional group. Therefore, the goal was to develop a separation, which could quantify all four potential isomers. For the pentafluoro analog, a normal phase screening across Daicel's library of immobilized chiral stationary phases (CSPs), yielded a very nice resolution on CHIRALPAK® IA-3 (Figure 2). No additional optimization from this initial screening was required.

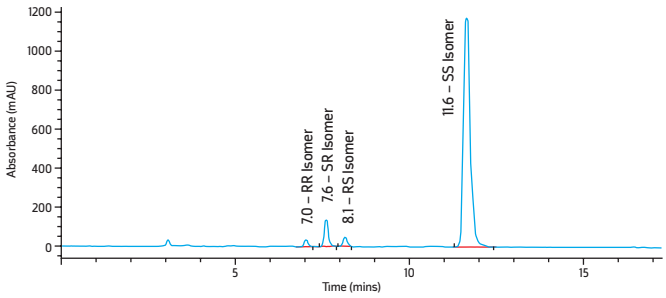

Figure 2: Separation of Remdesivir Pentafluoro Phosphoramidite KSM

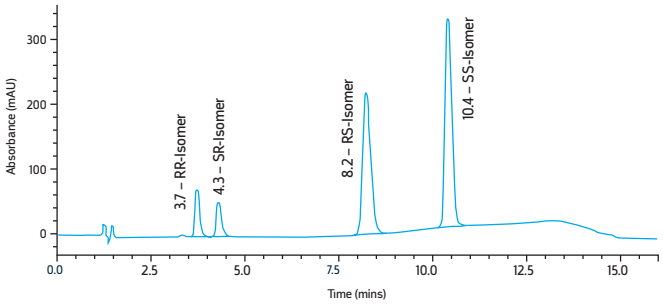

Figure 3: Separation of Remdesivir 4-Nitro Phosphoramidite KSM

The 4-nitro analog was a bit more challenging, as no viable separations on normal phase were initially found. However reversed-phase screening using a water/MeOH gradient yielded a partial separation for 2 of the 4 expected peaks. The addition of acetonitrile as a mobile phase component resulted in better selectivity and thus a separation of the final two, earlier eluting isomers (Figure 3).

The separation of the triol and tri o-benzyl nitrile starting materials is a chiral/achiral separation, which can be accomplished on a Daicel polysaccharide CSP. However in this case, Daicel's HILIC DCpak® PTZ column was used. The initial screening was performed without the addition of any additives, however the peak shape was less than desirable. The addition of 0.01% o-phosphoric acid to the mobile phase A component had a significant improvement to the peak shape, and optimization of the gradient lead to a baseline resolution of all four isomers (Figure 4).

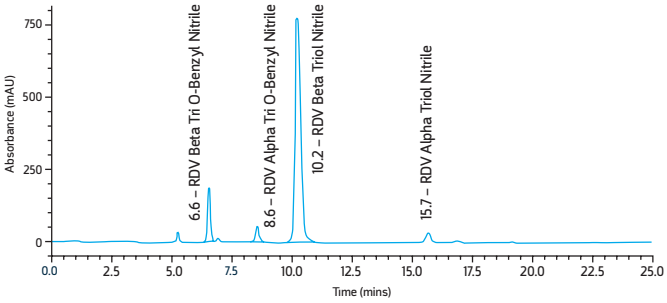

Figure 4: Separation of Remdesivir Triol Nitrile and Tri O-Benzyl Nitrile KSM

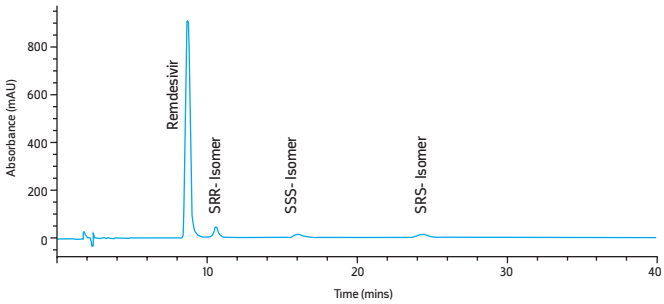

Figure 5: Separation of Remdesivir

Lastly, Remdesivir was screened and a good separation under normal phase conditions was observed, with slightly undesirable peak shape. Because the compound contains both basic and slightly acidic functional groups, the addition of both ethanolamine and formic acid was able to sharpen the peaks, and the combination of isopropanol and ethanol gave a nice compromise between selectivity and retention.